CLAIMS

WHAT IS CLAIMED IS:

1. A method of amplifying DNA, the method comprising:

(a) providing a single reaction mixture comprising:

(i) a DNA sample;

(ii) a first primer comprising a random sequence of nucleotides at its 3' end and a generic sequence 5' of the random nucleotides; and

(iii) a second primer comprising the generic sequence of the first primer and lacking the random sequence of the first primer;

(b) subjecting the DNA sample to DNA amplification by a first DNA polymerase wherein the first primer anneals to the DNA to allow the first DNA polymerase to produce a DNA product; and

(c) subjecting the DNA product of step (b) to DNA amplification by a heat-stable DNA polymerase wherein the second primer anneals to the DNA product.

2. The method of claim 1, wherein the DNA amplification of step (b) comprises the steps of denaturing the DNA product; annealing the first primer with the DNA to allow the formation of a DNA-primer hybrid; and incubating the DNA-primer hybrid to allow the first DNA polymerase to synthesize a DNA product.

3. The method of claim 2, wherein the DNA amplification steps are repeated at least one time.

4. The method of claim 1, wherein the DNA amplification of step (c) comprises the steps of denaturing the DNA product; annealing the second primer with the DNA product to allow the formation of a DNA-primer hybrid; and incubating the DNA-primer hybrid to allow the heat-stable DNA polymerase to synthesize a second DNA product.

5. The method of claim 4, wherein the second DNA product is flanked by the generic sequence and reverse complement of the generic sequence.

6. The method of claim 4, wherein the annealing temperature is higher than the optimal annealing temperature of the random sequence of nucleotides of the first primer.

7. The method of claim 4, wherein the DNA amplification steps are repeated about 30 to about 40 times.

8. The method of claim 1, wherein the first DNA polymerase has 5' to 3' exonuclease activity.

9. The method of claim 1, wherein said first DNA polymerase has primer displacement activity.

10. The method of claim 1, wherein the heat-stable DNA polymerase is Taq DNA polymerase.

11. The method of claim 1, wherein the first DNA polymerase and the heat-stable DNA polymerase are the same DNA polymerase.

12. The method of claim 1, wherein the first primer comprises about 4 to about 8 random nucleotides at its 3' end.

13. The method of claim 1, wherein the generic sequence of the first primer is about 15 to about 28 nucleotides in length.

14. The method of claim 1, wherein the random nucleotides of the first primer are G:C rich.

15. The method of claim 1, wherein the random nucleotides of the first primer are A:T rich.

16. The method of claim 1, wherein the generic sequence of the first primer comprises a single or multiple restriction enzyme recognition site.

17. The method of claim 1, wherein the DNA sample is selected from the group consisting of genomic DNA, microdissected chromosome DNA, yeast artificial chromosome (YAC) DNA, cosmid DNA, phage DNA, P1 derived artificial chromosome (PAC) DNA, and bacterial artificial chromosome (BAC) DNA.

18. The method of claim 1, wherein the DNA sample is selected from the group consisting of mammalian DNA, plant DNA, yeast DNA, viral DNA, and prokaryotic DNA.

19. The method of claim 1, wherein the DNA sample is obtained from a human, bovine, porcine, ovine, equine, rodent, avian, fish, shrimp, plant, yeast, virus, or bacteria.

20. The method of claim 1, wherein the DNA sample is bovine DNA.

21. The method of claim 1, wherein the DNA sample is tissue on a solid medium.

22. The method of claim 1, wherein the DNA sample is obtained from a buccal swab, a nose swab, blood, cord blood, amniotic fluid, embryonic tissue, hair, endothelial cells, hoof clippings, or fingernail clipping.

23. The method of claim 1, further comprising genotype analysis of the amplified DNA product.

24. The method of claim 1, further comprising identifying a single nucleotide polymorphism (SNP) in the amplified DNA product.

25. The method of claim 24, wherein the SNP is identified by Oligonucleotide Ligation Assay (OLA), Doublecode OLA, sequencing, Single Base Extension Assay, allele specific primer extension, or mismatch hybridization.

26. A method of amplifying DNA, the method comprising:

(a) providing a single reaction mixture comprising:

(i) a DNA sample;

(ii) a first primer comprising a random sequence of nucleotides at its 3' end and a generic sequence 5' of the random nucleotides;

(iii) a second primer comprising the generic sequence of the first primer and lacking the random sequence of the first primer; and

(iv) a heat-stable DNA polymerase;

(b) subjecting the DNA sample to DNA amplification wherein the first primer anneals to the DNA to allow the heat-stable DNA polymerase to produce a DNA product; and

(c) subjecting the DNA product of step (b) to DNA amplification wherein the second primer anneals to the DNA product.

27. The method of claim 26, wherein the heat-stable DNA polymerase is Taq DNA polymerase.

28. The method of claim 26, wherein the DNA amplification of step (b) comprises the steps of denaturing the DNA product; annealing the first primer with the DNA to allow the formation of a DNA-primer hybrid; and incubating the DNA-primer hybrid to allow the heat-stable DNA polymerase to synthesize a DNA product.

29. The method of claim 28, wherein the DNA amplification steps are repeated at least one time.

30. The method of claim 26, wherein the DNA amplification of step (c) comprises the steps of denaturing the DNA product; annealing the second primer with the DNA product to allow the formation of a DNA-primer hybrid; and incubating the DNA-primer hybrid to allow the heat-stable DNA polymerase to synthesize a second DNA product.

31. The method of claim 30, wherein the annealing temperature is higher than the optimal annealing temperature of the random sequence of nucleotides of the first primer.

32. A method of identifying a polymorphism , the method comprising:

(a) providing a single reaction mixture comprising:

(i) a DNA sample;

(ii) a first primer comprising a random sequence of nucleotides at its 3' end and a generic sequence 5' of the random nucleotides; and

(iii) a second primer comprising the generic sequence of the first primer and lacking the random sequence of the first primer;

(b) subjecting the DNA sample to DNA amplification by a first DNA polymerase wherein the first primer anneals to the DNA to allow the first DNA polymerase to produce a DNA product;

(c) subjecting the DNA product of step (b) to DNA amplification by a heat-stable DNA polymerase wherein the second primer anneals to the DNA product to allow the heat-stable DNA polymerase to produce amplified DNA products; and

(d) analyzing the amplified DNA products of step (c) to identify a polymorphism.

33. The method of claim 32, wherein the polymorphism is a single nucleotide polymorphism (SNP).

34. The method of claim 33, wherein the SNP is identified by Oligonucleotide Ligation Assay (OLA), Doublecode OLA, sequencing, Single Base Extension Assay, allele specific primer extension, or mismatch hybridization.

35. A method of amplifying DNA, the method comprising:

(a) providing a reaction mixture comprising:

(i) a DNA sample to be amplified;

(ii) a first primer comprising a random sequence of nucleotides at its 3' end and a generic sequence 5' of the random sequence; and

(iii) a second primer comprising the generic sequence and lacking the random sequence of the first primer;

(b) heating the reaction mixture to a temperature that denatures the DNA to be amplified;

(c) cooling the reaction mixture to a temperature that allows the random sequence of the first primer to hybridize to its complement DNA and incubating the reaction mixture to allow synthesis of a DNA product by a DNA polymerase;

(d) repeating steps (b) and (c) at least one time; and

(e) performing a series of DNA amplification reactions wherein the annealing step is at a temperature that selects for the generic sequence of the second primer hybridizing to complement DNA in the DNA product

over the random sequence of the first primer hybridizing to complement DNA in the DNA product.

36. In a method for amplifying a DNA sample on a solid medium, the improvement comprising precipitating the DNA sample on the solid medium and subjecting the precipitated DNA to DNA amplification to produce amplified DNA products.

37. The method of claim 36, wherein the solid medium is filter paper.

38. The method of claim 37, wherein the filter paper is chemically treated.

39. The method of claim 37, wherein the DNA sample is dehydrated on the filter paper.

40. The method of claim 36, wherein the DNA sample is precipitated with salt and alcohol, and rinsed with alcohol.

41. The method of claim 40, wherein the salt is selected from the group consisting of sodium acetate, potassium acetate, ammonium acetate, sodium chloride, and potassium chloride.

42. The method of claim 40, wherein the alcohol is ethanol or isopropanol.

43. A method of identifying a polymorphism, the method comprising:

(a) precipitating a DNA sample on a solid medium;

(b) providing a single reaction mixture comprising:

(i) the DNA sample;

(ii) a first primer comprising a random sequence of nucleotides at its 3' end and a generic sequence 5' of the random nucleotides; and

(iii) a second primer comprising the generic sequence of the first primer and lacking the random sequence of the first primer;

(c) subjecting the DNA sample to DNA amplification by a first DNA polymerase wherein the first primer anneals to the DNA to allow the first DNA polymerase to produce a DNA product;

(d) subjecting the DNA product of step (c) to DNA amplification by a heat-stable DNA polymerase wherein the second primer anneals to the DNA

product to allow the heat-stable DNA polymerase to produce a second DNA product; and

(e) analyzing the second DNA product to identify a polymorphism.

44. The method of claim 43, wherein the DNA sample is precipitated with salt and alcohol, and rinsed with alcohol.

45. A method of amplifying DNA, the method comprising:

(a) providing a reaction mixture comprising:

(i) a DNA sample;

(ii) a first primer comprising a random sequence of nucleotides at its 3' end and a generic sequence 5' of the random nucleotides; and

(iii) a second primer comprising the generic sequence of the first primer and lacking the random sequence of the first primer;

(b) subjecting the DNA sample to DNA amplification by a first DNA polymerase wherein the first primer anneals to the DNA to allow the first DNA polymerase to produce a DNA product; and

(c) subjecting the DNA product of step (b) to DNA amplification by a heat-stable DNA polymerase wherein the second primer anneals to the DNA product.

46. A method of amplifying DNA, the method comprising:

(a) providing a reaction mixture comprising:

(i) a DNA sample;

(ii) a first primer comprising a random sequence of nucleotides at its 3' end and a generic sequence 5' of the random nucleotides;

(iii) a second primer comprising the generic sequence of the first primer and lacking the random sequence of the first primer; and

(iv) a heat-stable DNA polymerase;

(b) subjecting the DNA sample to DNA amplification wherein the first primer anneals to the DNA to allow the heat-stable DNA polymerase to produce a DNA product; and

(c) subjecting the DNA product of step (b) to DNA amplification wherein the second primer anneals to the DNA product.